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## FINAL REPORT

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**EVALUATION OF ALTERNATIVE METHODS FOR INOCULATING ALFALFA**

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**Project Name:** Evaluation of alternative methods for inoculating alfalfa

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**(a) Abstract/Summary**

Granular *Rhizobium* inoculants for alfalfa were compared to pre-inoculated seed treatment and on-site inoculation with peat-based inoculants for their effect on stand vigor, productivity, N-fixation and quality in a three year field study and complimentary growth chamber study. Overall, the granular inoculant treatments were not superior to the traditional inoculant treatments, except in terms of producing slightly more biomass in the establishment year at two of the three sites. There were no differences in N-fixation parameters nor quality. Given the higher cost of granular inoculants, it is unlikely that they would offer an economic alternative to traditional inoculants.

**(b) Executive Summary**

Granular inoculant formulations are increasing in popularity as *Rhizobium* inoculants for pulse crops. They tend to be easier to handle and apply than liquid or peat-based inoculants, and there is some evidence in pulses that in first time pulse crops (i.e., fields that have never been seeded to a pulse) that they result in better nodulation, N-fixation and sometimes higher seed yields. Granular formulations are not widely available as inoculants for forage legumes like alfalfa. In theory, they should work as well, if not better in forage legumes than in pulse crops. Forage legumes have extremely small seeds which means that seed must place very shallowly in the soil. Granular inoculants enable the farmer to place the inoculant deeper in the soil than the seed, where it would be protected from harsh conditions of the atmosphere. Furthermore, if a producer wants to

apply a higher rate of inoculant, this can be done independently of increasing the seeding rate. In the study reported on here, the effectiveness of granular *Rhizobium* inoculant formulations for alfalfa production was compared to pre-inoculation of seed and on-site inoculation with a peat-based formulation at three field sites in the Black and Grey soil zones. In addition, different placements of the granular inoculant into the soil were investigated for their effect on early N-fixation and biomass yield in a greenhouse study. Two of the three field sites (Conservation Learning Centre and Seager Wheeler) were established as hay production sites, where oat was grown as the cover crop, and the third site as a processed 'dehy' site, where canola was grown as the cover crop. Five inoculation treatments were established at each site: uninoculated control, pre-inoculated alfalfa seed, on-site peat-inoculated seed, granular inoculant seed-placed with the alfalfa seed, and granular inoculant side-banded into the soil. Alfalfa stand counts, weed surveys, nodulation scoring, measurement of N-fixation, and forage harvests were performed at all of the sites. In the establishment year (1999) one forage cut was taken; in the two production years of the study (2000 & 2001) two cuts were taken.

Overall, the granular inoculants were not superior to the pre-inoculated seed treatment or the peat-inoculant treatment. In the establishment year the banded granular treatment did give higher yields than uninoculated controls at all of the sites and was among the highest yielding of all of the inoculant treatments. The higher yields was related to slightly higher stand counts in these treatments at two of the sites. However, by the first production year differences in productivity among the treatments had disappeared.

In the growth chamber study the different placements of the inoculants did not affect the numbers and sizes of biologically active nodules (nodulation scores) but did affect where the nodules occurred on the root system. Placing the granules below the seed tended to cause nodulation on the bottom portion of the root system; placing the granules with the seed caused nodules to be spread evenly throughout the root system and direct inoculation of the seed caused more nodules to form in the crown rather than on lateral roots. However, in neither the growth chamber study nor the field study did differences in nodulation reflect percent nitrogen derived from fixation.

A serious drought developed in the second production year (2001). The drought did cause treatment differences that were apparent in the establishment year to reappear in

the second production year at the CLC site. There were no differences in N-fixation, however there were differences in yield. There was very poor stand establishment at this site. However in the first production year, where soil moisture was adequate, the small numbers of plants were very productive and compensated for the low numbers of plants. However, in the second production year the drought severely limited biomass production and could not be compensated for by the small numbers of plants growing. However, the differences among treatments seemed to be related more to the seeding equipment than the inoculation treatment per se. There seemed to be a problem with the seed-placed or seed-applied treatments flowing through the hoses of the seeder.

Overall, the granular inoculant formulations were not better than the traditional, pre-inoculated seed or the on-site applied peat inoculants. In the establishment year, there was some evidence of superior performance with the granular inoculants, however, it is unlikely that the small increases in productivity are enough to justify the double to triple cost of the granular formulations compared to the seed applied products. The higher cost might be justified with biennial crops like the clovers, where the crop is only grown for two years.

One somewhat surprising observation from the study is that uninoculated controls were generally as good as the inoculant treatments in both the growth chamber and field studies. It seems that indigenous Rhizobia frequently occur in high enough numbers to adequately inoculate a crop. Not only did nodules form but in general N-fixation and biomass production also were comparable to inoculation treatments. Research needs to determine the conditions controlling rhizobial survival, or develop some means for a farmer to determine whether inoculation is likely to be worthwhile (i.e., some sort of soil bioassay). Although there have been numerous reports of uninoculated stands producing and fixing N as well as inoculated stands, there are also an equivalent number of reports of inoculant failure and questions about post-emergent inoculation of forage legume stands. It is clear too premature to recommend producers not inoculate forage legume crops.

**(b) Technical Report:**

**Introduction:**

*Rhizobium* inoculants for alfalfa production typically are delivered as coatings on pre-inoculated seeds or as a peat-based powder formulation. Pre-inoculated seed is very convenient, requiring no additional operation at the farm level, but is highly susceptible to loss of viability of the applied rhizobia. Peat-based inoculant applied with a sticker can be very messy and time-consuming when the producer's time is at a premium. In recent years, manufacturers have begun to develop and formulate *Rhizobium* inoculants as granules. In the granular form, the *Rhizobium* inoculant is applied to the soil instead of the seed, in the same manner as fertilizer, and can be applied before, during or after the seeding operation. Granular formulations are increasing in popularity as inoculants for pulse crops. In several studies, granular inoculants lead to improved nodulation and higher seed yields compared to traditional formulations (Bezdicek et al., 1978; Muldoon et al., 1980). Similarly, in a study performed in Saskatchewan, confirmed that granular inoculant formulations applied in the seed row encouraged better nitrogen fixation and higher yields in chickpea and bean compared to the peat based or liquid counterparts (Kyei-Boahen, 2002).

Inoculation of alfalfa (and clovers) with granular formulations may prove beneficial for a number of reasons. Because of its very small seed size, alfalfa must be planted very shallowly in the soil (maximum depth of ¾ inch, depending on soil conditions). Placement of the inoculant below the seed row should protect rhizobia from temperature and moisture fluctuations (Smith, 1992). In addition, the small seed size limits the number of rhizobia that can be attached to the seed coat (Clayton et al., 1996). While this may be overcome by the large seed numbers that are planted, placement of granules should prove effective in increasing should prove effective in increasing numbers of viable rhizobia in contact with the root system because the rate of granule placement can be regulated. Placement of rhizobia below the seed ensures that the rhizobia are favorably place in relation to the roots.

Little information is available about the effective use and application of granular inoculants in forage legumes. To our knowledge no one has ever investigated the

possibility of using the companion crop seed as a delivery system for inoculant. Information is needed about the efficacy of granular inoculants as compared to more traditional inoculant formulations. Ease of application, effectiveness of nodulation and cost of inoculation are all factors that must be considered.

The overall objectives of this research is to evaluate the usefulness of granular inoculants for alfalfa production. Specific objectives are:

(i) to investigate the impact of inoculant placement on nodulation and early nitrogen fixation in alfalfa

(ii) to compare the efficacy of granular inoculation to traditional peat-based inoculation of alfalfa.

**Field Study:**

***Site Establishment and Management:***

Three field sites were established in the Spring of 1999: one site at the Seager Wheeler farm near Rosthern, a second site at the Conservation Learning Centre (CLC) near Prince Albert, and a third site on producer's land near Tisdale. The three field sites provide a range of soils and topography typical of alfalfa production. Selected sites had low soil N values (< 20 kg/ha) and had not been seeded to alfalfa in the past five years. These sites were maintained and sampled a total of three years; 1999, 2000 and 2001.

**Tisdale:** This was established as a processed "dehy" production site using canola (46A73; Pioneer Hi-Bred Production Ltd.) as the cover crop. The site had been seeded to flax in 1998 and represented a conventionally tilled site. Experimental design was a randomized complete block design with four replications. Individual treatment plots were 2.6-m x 11-m. Plots were seeded using a disc seeder with 8-inch row spacing. The seeder was equipped with two boxes and a set of cones. All of the plots received the same number of passes with the seeding equipment.

Treatments consisted of: 1, un-inoculated *alfalfa (Medicago sativa* cv. Beaver; this variety was used for all of the inoculated treatments) – control; 2, pre-inoculated alfalfa; 3, on-site inoculated alfalfa (peat-based inoculant applied at seeding); 4, alfalfa + granular inoculant side-banded; 5, granular inoculant placed with the alfalfa seed. Two

additional treatments were included to be used as reference crops for quantifying $N_2$-fixation using the $^{15}N$ dilution technique: an ineffectively nodulating alfalfa that does not fix N (*Medicago sativa* cv. Saranac) and smooth brome grass. Because the Saranac alfalfa was developed in the U.S. (University of Minnesota) we were not sure of its ability to survive the winters in Saskatchewan, so included smooth brome grass as an alternative reference crop. Smooth brome grass plots were hand-broadcast and raked in. All plots were seeded with canola as the cover crop.

The pre-inoculated seed, peat-based and granular inoculants were prepared by MicroBioRhizogen Corp. (Saskatoon, SK) using identical strains of *R. meliloti* bacteria. Alfalfa was seeded at a rate of 7 lb acre$^{-1}$; canola at 5 lb acre$^{-1}$; and smooth brome at 9.5 lb acre$^{-1}$. The granular inoculant was applied at a rate of 7 lb acre$^{-1}$. Plots were seeded in two passes. The canola and phosphate fertilizer (20 lb acre$^{-1}$, 12-54-0) were placed in the first pass. Canola was placed approximately ¼ inch deep and phosphate 1 inch to the side and 1 inch below the seed. Alfalfa seed and the inoculant were placed in the second pass. Alfalfa was seeded approximately ¼ inch deep. Depending on the treatment, granular inoculant was placed with the alfalfa seed or side-banded at the depth of the fertilizer.

Plots were sprayed post-emergently with Pursuit according to manufacturer's recommendations.

**Seager Wheeler:** This site was seeded May 26$^{th}$, 1999 and was established as a hay production site, using oat (*Avena sativa*) as the cover crop. The site had been seeded to a triticale/pea mix in 1998 and represented a conventionally tilled site. The experimental design, plot size and seeding equipment were the same as used at the Tisdale site. The same treatments as the Tisdale site were seeded except that oat was seeded instead of canola as the cover crop. Oat was seeded at a rate of 40 lb acre$^{-1}$. Plots were seeded in two passes. In the first pass the oat and phosphate fertilizer (20 lb acre$^{-1}$, 12-54-0) were placed. Oat was placed approximately 1 inch deep and the phosphate fertilizer 1 inch to the side of the seed. Alfalfa seed and the inoculant were placed in the second pass. Alfalfa was seeded approximately ¼ inch deep. Depending on the treatment, granular inoculant was placed with the alfalfa seed or side-banded at the depth of the fertilizer/oat.

Plots were sprayed post-emergently with Pardner according to the manufacturer's recommendations.

**Conservation Learning Centre:** This site was seeded June 2nd, 1999. Like the Seager Wheeler site, this site was established as a typical hay production site, using oat as the cover crop. The site had been seeded to barley in 1998 and represented a minimum till site. Several weeks before seeding the field was sprayed with Roundup transorb. Alfalfa and oat were seeded directly into the barley stubble. Plots were seeded using an Edwards hoe drill with 8 inch row spacing. The size of individual treatment plots was 4-m x 11-m. The seeder was equipped with two split boxes. The treatments including seeding rates, and seed and fertilizer placements were the same as those used at the Seager Wheeler site. Plots were seeded in two passes. The oat seed and phosphate fertilizer (20 lb $acre^{-1}$, 12-54-0) were placed in the first pass. Alfalfa seed and inoculant were placed in the second pass. Depending on the treatment, granular inoculant was placed with the alfalfa seed or side-banded at the depth of the fertilizer/oat.

Post-emergent herbicides were not applied at this site.

**All Sites:** No herbicides were applied during the 2000 and 2001 growing seasons. All of the sites were harvested for forage yield once in 1999 (establishment year) and twice in 2000 and 2001 (production years). The first cut was taken in late June and the second cut in late August at all of the sites in both years.

***Soil Sampling:***

Prior to seeding in 1999 and during the first week of May in 2000 and 2001, soil samples were collected to a 30 cm depth. Composite (10 samples per field) soil samples were collected from each of the sites and analysed for N, P, K, pH, and E.C. by EnviroTest Laboratories, Saskatoon.

***Biological $N_2$ fixation:***

Two to three weeks after seeding $^{15}N$-labelled $NH_4NO_3$ (3.75 lb $acre^{-1}$ 13.3 atom %) was applied to 1-$m^2$ microplots within each treatment plot. Application of the fertilizer was followed with an equal volume of water to wash the fertilizer from the foliage into the soil. Subsamples of shoot tissue were harvested from these microplots 8 weeks later.

Nitrogen-15-labelled-$NH_4NO_3$ was reapplied to different 1-$m^2$ microplots during the first week of May in 2000 and 2001. Shoots from the microplots were subsampled on the same day that the plots were harvested for first cut forage yield. In 2000, a second application of $^{15}N$-labelled $NH_4NO_3$ was applied after the first cut was harvested. There was a lot of rainfall early in the growing season in 2000 and it was thought that the first application of N fertilizer might be leached out of the rooting zone. Shoot subsamples were collected on the day of the second cut in 2000 and 2001.

Shoot tissues were oven dried and ground. These samples were analysed by mass spectrometery for $^{15}N$ content and the amount of N derived from atmosphere quantified.

***Fibre Analyses*:**

Forage fiber analyses were conducted using the procedure outlined by Goering and Van Soest (1975). The procedure separates neutral-detergent fibre (NDF), and acid-detergent fibre (ADF). Neutral detergent fibre is a method for analysing the total fibre content in plant feed, and ADF measures the lignocellulose component in feed and is essentially the insoluble fibre component. Fibre analyses were performed in the first year only because no differences were detected among treatments in this first year.

***Sampling:***

A weed survey and alfalfa stand count were conducted mid-season at each of the sites in all three years. Weed species in a $m^2$ area within each treatment plot were identified and counted, along with the number of alfalfa plants. The $m^2$ sampling frames were positioned to cover the same number of alfalfa rows in each treatment.

At this same sampling time root/nodule samples were recovered and returned to the laboratory for analysis. Intact roots of five plants per treatment plot were dug up with a shovel. Roots were washed free of soil and nodulation scored (Table 1). Three people scored each of the root samples independently and the average of these scores was used to calculate the averages for a site. Root samples were collected in 1999 and 2000. Sampling was attempted in 2001 but the extremely dry soil conditions and the deep rooting of the plants made collecting intact root systems impossible.

A $m^2$ subsample was hand-harvested from each of the treatment plots at each of the harvest periods (one cut in 1999, and two cuts in 2000 and 2001). These $m^2$ harvest samples were oven dried and weighed. After the $m^2$ harvest samples were removed, the remainder of the aboveground biomass was removed from the plots.

**Table 1. Nodulation scoring for alfalfa. Assessment based on 5 plants/sample.**

| No. plants with pink nodules | Total no. of nodules | Score |
|---|---|---|
| >3 plants | >30 | 5 |
| >3 plants | 10-30 | 4 |
| >3 plants | 5-10 | 3 |
| >3 plants | <5 | 2 |
| 1-3 plants | <5 | 1 |
| 0 plants | | 0 |

**Growth Chamber Experiment:**

A growth chamber experiment examined the impact of inoculant placement on subsequent nodulation and early nitrogen fixation in alfalfa. Two alfalfa genotypes were tested: Beaver representing a commonly grown taproot type of alfalfa, and Rambler representing a commonly grown fibrous root type of alfalfa. The experiment consisted of the following five inoculant treatments using the two genotypes: uninoculated control; a self-sticking peat-based *Rhizobium* inoculant seed applied; a granular *Rhizobium* inoculant placed with the alfalfa seed; a granular *Rhizobium* inoculant placed 2.5 cm below the seed; a granular *Rhizobium* inoculant placed 5.0 cm below the seed. Pots containing ineffectively nodulating alfalfa (cv. Saranac) and smooth brome grass were included as reference crops for the $^{15}N$ dilution technique for quantifying N-fixation. Plants were destructively sampled at 4, 8 and 12 weeks after planting. Four replicates of each treatment were sampled on each of the dates.

Soil testing low in nitrogen was collected from a farmers field near Saskatoon. The soil was mixed in a 2:2:1 ratio of soil:sand:vermiculite (vol:vol:vol). This mixture was placed in 150 cm diameter pots and the pots seeded with the appropriate treatments.

Sixteen alfalfa seeds were planted per pot and thinned back to eight seedlings per pot post-emergently. This final seeding rate corresponded to a rate of 8 kg $ha^{-1}$ (ca. 7 lb $acre^{-1}$) and was comparable to the rate used in the field study. The different depths of inoculant placement were achieved by first wetting the soil mixture and then punching 16 holes to the depth of the inoculant placement (either 2.5 or 5.0 cm). Two or three inoculant granules were placed in the bottom of the hole. Soil mixture was added to fill the holes. The alfalfa seed was then placed approximately 0.5 cm (1/4) inch from the soil surface. Shortly after the seedlings had emerged and were thinned, $^{15}N$-labelled $NH_4NO_3$ (0.028g 10 atom % $NH_4NO_3$ per pot) was applied to the pots.

At each of the sampling dates, plants were harvested at ground level. Shoots were oven dried and the soil containing the roots and nodules bagged in plastic and stored in a cold room (4°C) until the roots could be washed, scored and oven dried (a maximum of 5 days). Nodulation was scored using the same procedure outlined for field samples. Shoot and root samples were ground and analysed by mass spectrometry analysis of $^{15}N$ content.

**Results and Discussion:**

**Establishment year (1999):**

Inoculation treatment affected nodulation scores only at the Seager Wheeler site, where plants inoculated with the granular seed placed inoculant had the lowest scores and plants from pre-inoculated seed had the highest score (Table 3). Plants in the control plots had nodulation scores as good as or better than the inoculated treatments. One shortcoming of the nodulation scoring system is that it does not incorporate any measure of where on the root system the nodules occur. There is some evidence in pulse crops that nodules located on the lateral roots are more effective at fixing nitrogen than nodules located on the tap root (Kyei-Boahen et al., 2002). In this study nodulation scores do not accurately predict biological nitrogen fixation (%ndfa) (Table 3). Plants at the Seager Wheeler site showed no difference in %ndfa. In contrast, at Tisdale, no difference in nodulation score was observed associated with the inoculation treatments, however, %ndfa did show a treatment response. At this site the control plants had lower %ndfa

than plants in all of the treatments except the granular banded treatment. At the CLC site there were no differences in nodulation or %ndfa among plants inoculated with the different treatments.

Differences in alfalfa stand establishment were detected only at the CLC site (Table 3). Establishment was extremely poor at this site in all of the treatments where the inoculants were seed placed. Establishment in the banded granular inoculant treatment was comparable to the check plots. The poor stand counts associated with the seed placed inoculant treatments at the CLC site translated into lower biomass production in these same treatments (Table 3). The poor alfalfa establishment at the CLC site may be because this site is a minimum till site, where the seed and inoculant were placed directly in the stubble. Although the seedbed was quite firm, it was not particularly smooth and seed and inoculant may have had a tendency to lie on the soil surface. Additionally, there seemed to be problems with the peat-based products (pre-inoculated seed and the on-site peat inoculated seed) not flowing properly through the hoses supplying the boots of the seeder.

At all three of the sites the banded granular treatment was among the most productive (Table 3). At Seager Wheeler and Tisdale, all of the treatments showed good productivity. At Tisdale, the on-site inoculated treatment resulted in the poorest biomass production of all the treatments, whereas at Seager Wheeler, the pre-inoculated seed and seed-placed granular treatment were the poorest. However, unlike the CLC site all of the treatments at Tisdale and Seager Wheeler were at least as good as the uninoculated control.

Neutral and acid detergent fibre analyses provide a measure of forage fibre quality. Acid detergent fibre is the highly indigestible parts of the forage (composed of lignin, cellulose, silica and insoluble forms of nitrogen), and neutral detergent fibre is a partially digestible fraction (composed mainly of cell walls). None of the inoculation treatments affected either of these fibre components (Table 4). Because no differences were found in the establishment year, this measurement was not included in subsequent sampling.

Inoculation treatment had little affect on the weed populations in the establishment year at any of the sites (Table 5). Grassy weeds were of little significance at any of the sites, occurring in very small numbers. Only at Seager Wheeler was any difference in

weed densities observed ($P<0.100$) and was attributed to differences in broadleaf weeds. At this site plots seeded with pre-inoculated seed had the lowest weed densities, and plots where seed was on-site inoculated had the highest weed densities. These differences may have been due to spatial variability in weed populations rather than a treatment affect. Weeds tend to have very patchy distributions fields so that different parts of a field are inherently much more affected by weeds than other parts of the same field. Considering that alfalfa in the pre-inoculated treatment at the Seager Wheeler site had very average alfalfa stand counts, compared to other treatments at the same site, and among the lowest biomass production, it does not appear that the alfalfa in the pre-inoculated treatment was more competitive with weeds. There was no relationship between weed densities and alfalfa stand at any of the sites (Tables 3 & 5). Although a statistically significant difference ($P<0.100$) in grassy weed density was observed at the CLC site, this was due to the fact that only one of the treatment plots (granular inoculant seed-placed) had any grassy weeds at all.

**Table 2.** Physical and chemical characteristics of field soils and growth chamber soil sampled in the spring of each year of the study.

| Site-year | Texture | pH | E.C. (mS/cm) | $NO_3$-N (lb/acre) | P (lb/acre) | K (lb/acre) | S (lb/acre) |
|---|---|---|---|---|---|---|---|
| CLC-99 | Clay Loam | 6.9 | 0.4 | 14 | 13 | 1080 | 34 |
| CLC-00 | Clay Loam | 7.0 | 0.2 | 37 | 24 | 1080 | 68 |
| CLC-01 | Clay Loam | 6.7 | 0.2 | 31 | 28 | 984 | 49 |
| SW-99 | Loam | 6.6 | 0.2 | 21 | 24 | 880 | 38 |
| SW-00 | Loam | 7.0 | 0.2 | 37 | 24 | 1080 | 68 |
| SW-01 | Loam | 6.7 | 0.2 | 31 | 28 | 984 | 45 |
| Tis-99 | Clay Loam | 7.2 | 0.4 | 16 | 16 | 572 | 40 |
| Tis-00 | Clay Loam | 7.5 | 0.5 | 25 | 20 | 620 | 54 |
| Tis-01 | Clay Loam | 7.5 | 0.5 | 26 | 37 | 620 | 48 |
| GrthCh | Loam | 7.0 | 0.3 | 14 | 27 | 740 | - |

**Table 3.** Yield and biological nitrogen fixation parameters for alfalfa in the establishment year (1999) inoculated with different *Rhizobium* formulations.

| Treatment | CLC | SW | Tisdale | CLC | SW | Tisdale |
|---|---|---|---|---|---|---|
| | Nodulation Score | | | %ndfa[1] | | |
| Control | 3.3 | 3.8ab | 2.5 | 53.5 | 37.2 | 36.6b |
| Pre-inoculated | 2.5 | 4.8a | 3.0 | 48.6 | 33.7 | 44.2a |
| On-site inoculated | 2.7 | 3.5b | 3.0 | 50.4 | 39.5 | 44.1a |
| Granular banded | 3.0 | 3.3b | 2.5 | 53.3 | 38.3 | 41.1ab |
| Granular seed-placed | 3.0 | 1.8c | 2.3 | 50.3 | 38.7 | 45.1a |
| *ANOVA* | | *Probability* | | | *Probability* | |
| Block | 0.6623 | 0.7145 | 0.1568 | 0.004 | 0.001 | 0.001 |
| Treatment | 0.7725 | 0.0022 | 0.1410 | 0.560 | 0.220 | 0.036 |
| | Biomass (g $m^{-2}$) | | | Alfalfa stand (No. $m^{-2}$) | | |
| Control | 28b | 33b | 35b | 21a | 28 | 22 |
| Pre-inoculated | 3c | 31b | 58a | 2b | 27 | 31 |
| On-site inoculated | 7c | 45a | 37b | 3b | 23 | 23 |
| Granular banded | 46a | 48a | 55a | 25a | 34 | 16 |
| Granular seed-placed | 4c | 38ab | 44b | 2b | 42 | 23 |
| *ANOVA* | | *Probability* | | | *Probability* | |
| Block | 0.174 | 0.224 | 0.024 | 0.174 | 0.632 | 0.461 |
| Treatment | 0.000 | 0.016 | 0.046 | 0.000 | 0.362 | 0.332 |

[1]Percent nitrogen derived from the atmosphere (biological nitrogen fixation)

**Table 4.** Fibre quality parameters for alfalfa in the establishment year (1999) inoculated with different *Rhizobium* formulations.

| Treatment | CLC | SW | Tisdale | CLC | SW | Tisdale |
|---|---|---|---|---|---|---|
| | %NDF[1] | | | %ADF[2] | | |
| Control | 38 | 39 | 38 | 27 | 28 | 26 |
| Pre-inoculated | 33 | 34 | 41 | 26 | 24 | 30 |
| On-site inoculated | 32 | 35 | 39 | 23 | 25 | 27 |
| Granular banded | 39 | 37 | 40 | 29 | 25 | 29 |
| Granular seed-placed | 37 | 38 | 40 | 27 | 27 | 29 |
| *ANOVA* | | *Probability* | | | *Probability* | |
| Block | 0.2480 | 0.3225 | 0.1899 | 0.1682 | 0.4719 | 0.1393 |
| Treatment | 0.3900 | 0.3403 | 0.5566 | 0.1280 | 0.3010 | 0.3093 |

[1]Percent neutral detergent fibre
[2]Percent acid detergent fibre

**Table 5.** Weed densities in the emergence year (1999) of alfalfa plots inoculated with different *Rhizobium* formulations.

| Treatment | CLC | SW | Tisdale |
|---|---|---|---|
| | ---------- Broadleaf weeds (no./$m^2$) ---------- | | |
| Control | 10 | 16ab | 2 |
| Pre-inoculated | 16 | 8b | 4 |
| On-site inoculated | 10 | 26a | 3 |
| Granular banded | 15 | 15ab | 12 |
| Granular seed-placed | 13 | 12ab | 11 |
| *ANOVA* | | *Probability* | |
| Block | 0.0088 | 0.1077 | 0.3475 |
| Treatment | 0.5158 | 0.0943 | 0.1890 |
| | ---------- Grassy weed (no./$m^2$) ---------- | | |
| Control | 0 | 1 | 0 |
| Pre-inoculated | 0 | 1 | 0 |
| On-site inoculated | 0 | 2 | 3 |
| Granular banded | 0 | 3 | 0 |
| Granular seed-placed | 1 | 3 | 0 |
| *ANOVA* | | *Probability* | |
| Block | 0.4199 | 0.5791 | 0.4199 |
| Treatment | 0.0815 | 0.4989 | 0.3418 |
| | ---------- Total weeds(no./$m^2$)[1] ---------- | | |
| Control | 10 | 17ab | 2 |
| Pre-inoculated | 17 | 9b | 4 |
| On-site inoculated | 10 | 28a | 6 |
| Granular banded | 15 | 18ab | 2 |
| Granular seed-placed | 14 | 15ab | 11 |
| *ANOVA* | | *Probability* | |
| Block | 0.017 | 0.1328 | 0.188 |
| Treatment | 0.548 | 0.0696 | 0.227 |

[1]May include non-vascular *Equisetum sp.* not counted as either broadleaf or grassy weeds.

**First Production Year (2000):**

Alfalfa at all of the sites over-wintered very well and showed excellent re-growth early in the spring of 2000 (Table 6). The CLC site in particular, where in 1999 emergence and establishment was very poor, showed good re-growth in 2000. Although the numbers of plants per $m^2$ was lower at the CLC site than the other two sites, productivity in terms of first cut biomass was not lower (Table 6). This was probably due, at least partially, to the fact that the CLC site was sampled two weeks later than the Seager Wheeler and Rosthern sites. None-the-less by the time of the second cut this site was producing more than the Tisdale site.

Treatment differences in nodulation observed at the Seager Wheeler site in 1999 (Table 3) were no longer apparent in 2000 (Table 6). In particular,, the nodulation of alfalfa inoculated with the granular seed-placed treatment had improved substantially. Nitrogen fixation measured at the time of the first cut of alfalfa was not affected by inoculation treatment. Even the control treatments, which were not inoculated, showed very high levels of N fixation. Only at the time of the second cut did control treatments have significantly lower %ndfa than most of the inoculation treatments at two of the three sites (CLC and Tisdale). None of the sites were established on land that had grown alfalfa for at least five years, although all were established in areas where alfalfa production is common. It appears that the soils have high enough indigenous levels of *Rhizobia* that are capable of nodulating and fixing N in symbiosis with alfalfa. This study represents only a very small study area, so cannot be generalized to other areas in Saskatchewan. However, over the past few years there have been more and more reports of nodulation and N fixation in uninoculated forage legume crops. An estimated 80 % of alfalfa producers in the Star City region of the province (Northeastern Saskatchewan) do not inoculate alfalfa (Lyle Cowell, personal communication). At the same time there continue to be reports of inoculant failure and questions about inoculating post-emergently, so clearly inoculation is necessary in many instances. Research into the factors that affect the requirement for alfalfa inoculation, and an inventory of indigenous *Rhizobium* levels in soils across the province is warranted. The reduced need to inoculate alfalfa may be because of the very mild winters experienced in much of Saskatchewan over the past several years. Perhaps the rhizobia are over-wintering in higher numbers.

In terms of biomass production for the first and second cuts, none of the treatments were consistently superior at all three sites (Table 6). At all of the sites, the control plots (uninoculated) were very productive, indicating that either the soil N supply was adequate to sustain growth or that native *Rhizobium* populations in the soil are sufficient. Soil sampling done in the spring of each year indicated low initial soil available N levels at all of the sites (Table 2). The granular inoculant placed with the alfalfa seed was the lowest producing treatment at the Tisdale site (Table 6). At the CLC, the on-site peat inoculated alfalfa seed treatment showed the lowest productivity. This also was observed in the 1999 field season (Table 3) and may have been caused by the peat inoculated seed not flowing properly through the seeder (Edward's hoe-drill). The negative effects at seeding appear to have continued in to the second year of production. However, in terms of nodulation, the on-site peat inoculation treatment showed the highest nodulation scores at all three sites in 2000. A high score is indicative of larger numbers of active (pink) nodules compared to a lower score. In the case of alfalfa, (unlike large seeded pulses) direct contact with the seed might encourage colonization and successful infection of the root system with the *Rhizobium*.

Although the statistical analysis performed does not allow direct comparisons between the sites, it is interesting to make some general observations about the percentage of N derived from fixation at the different sites. Percent Ndfa measured at the time of the second cut was 10-20% higher at the CLC site that at the Seager Wheeler and Tisdale sites. Water is required for biological $N_2$ fixation to occur. Although spring moisture was good in 2000, excessive soil drying was becoming an issue toward the end of the growing season. The higher %Ndfa at the CLC is probably because this site is a direct seeding/minimum tillage site that had more stored soil moisture than the other conventionally tilled sites. In 1999, the CLC was the poorest site for establishment, which was probably due to the seedbed characteristics at seeding, but once the plants established this site was at an advantage in terms of %Ndfa because of its soil water characteristics.

Overall, weeds were not much of a problem at any of the sites in 2000 (Table 7). Surprisingly volunteer oat (Seager Wheeler and CLC) and canola (Tisdale), the first year cover crops, did not pose a significant problem at any of the sites. Presumably, the early

establishment of the alfalfa stand effectively competed with any weeds and volunteer crops present. Weed populations were affected by inoculation treatment at the CLC and Seager Wheeler sites. As in 1999, in 2000 broadleaf weeds were more prevalent than grassy weeds at the CLC site. Pre-inoculated and control plots had the lowest weed numbers, and the on-site inoculated plots the highest. It appears that lower stand counts and higher weed densities were contributing to the lower yields from those plots inoculated on-site with peat-based inoculant at the CLC site. At the Seager Wheeler on-site inoculated plots again had the highest weed densities. At this site, grassy weeds were more prevalent than at the other sites. However, the greater numbers of weeds did not translate into lower stand counts or significantly lower yields at Seager Wheeler. Weeds simply were not a problem at Tisdale (Table 7).

**Table 6.** Productivity parameters and biological nitrogen fixation parameters in the first production year (2000) for alfalfa inoculated with different *Rhizobium* formulations.

| Treatment | CLC | SW | Tisdale | CLC | SW | Tisdale |
|---|---|---|---|---|---|---|
| | ------- First cut biomass ($g/m^2$) ------- | | | ------- Second cut biomass ($g/m^2$) ----- | | |
| Control | 295 | 307 | 185a | 282 | 292 | 177 |
| Pre-inoculated | 291 | 280 | 174ab | 277 | 268 | 168 |
| On-site inoculated | 158 | 259 | 161ab | 152 | 248 | 156 |
| Granular banded | 316 | 280 | 188a | 303 | 268 | 180 |
| Granular seed-placed | 274 | 273 | 152ab | 262 | 262 | 145 |
| *ANOVA* | | *Probability* | | | *Probability* | |
| Block | 0.0400 | 0.5929 | 0.2198 | 0.0413 | 0.5785 | 0.1036 |
| Treatment | 0.1238 | 0.4448 | 0.0776 | 0.1297 | 0.4810 | 0.2448 |
| | ---------- First cut %ndfa[1] ---------- | | | ---------- Second cut %ndfa[1] ---------- | | |
| Control | 86.8 | 71.3 | 84.0 | 86.2b | 68.3 | 61.9b |
| Pre-inoculated | 87.8 | 74.8 | 82.4 | 90.6a | 68.6 | 75.8a |
| On-site inoculated | 87.8 | 70.3 | 74.3 | 91.8a | 67.2 | 80.6a |
| Granular banded | 87.3 | 74.5 | 81.7 | 84.5b | 73.0 | 80.0a |
| Granular seed-placed | 87.2 | 71.7 | 77.9 | 90.5a | 70.0 | 74.4a |
| *ANOVA* | | *Probability* | | | *Probability* | |
| Block | 0.825 | 0.002 | 0.715 | 0.005 | 0.091 | 0.136 |
| Treatment | 0.760 | 0.610 | 0.799 | 0.062 | 0.712 | 0.064 |
| | -------- Stand counts ($no./m^2$) ------- | | | ---------- Nodulation score ---------- | | |
| Control | 68a | 110 | 104 | 2.8 | 2.6 | 3.0 |
| Pre-inoculated | 62ab | 100 | 111 | 2.8 | 3.3 | 3.0 |
| On-site inoculated | 28b | 96 | 105 | 3.6 | 3.6 | 3.4 |
| Granular banded | 62ab | 81 | 83 | 3.0 | 2.4 | 3.2 |
| | 44ab | 109 | 123 | 3.1 | 3.5 | 3.0 |
| *ANOVA* | | *Probability* | | | *Probability* | |
| Block | 0.4846 | 0.6935 | 0.6297 | 0.2290 | 0.0120 | 0.0052 |
| Treatment | 0.0944 | 0.3817 | 0.1164 | 0.6563 | 0.1076 | 0.7288 |

**Table 7.** Weed densities in the first production year (2000) of alfalfa plots inoculated with different *Rhizobium* formulations.

| Treatment | CLC | SW | Tisdale |
|---|---|---|---|
| | ---------- Broadleaf weeds (no./$m^2$) ---------- | | |
| Control | 5b | 7 | 1 |
| Pre-inoculated | 6b | 9 | 2 |
| On-site inoculated | 11a | 9 | 4 |
| Granular banded | 8ab | 7 | 5 |
| Granular seed-placed | 9ab | 8 | 5 |
| *ANOVA* | | *Probability* | |
| Block | 0.2445 | 0.0093 | 0.5018 |
| Treatment | 0.0420 | 0.9434 | 0.7856 |
| | ---------- Grassy weeds (no./$m^2$) ---------- | | |
| Control | 0 | 2b | 0 |
| Pre-inoculated | 0 | 5b | 1 |
| On-site inoculated | 2 | 26a | 0 |
| Granular banded | 0 | 10ab | 1 |
| Granular seed-placed | 1 | 13ab | 1 |
| *ANOVA* | | *Probability* | |
| Block | 0.2163 | 0.2144 | 0.1145 |
| Treatment | 0.5451 | 0.0585 | 0.8603 |
| | ---------- Total weeds (no./$m^2$) ---------- | | |
| Control | 5b | 9b | 1 |
| Pre-inoculated | 5b | 14b | 3 |
| On-site inoculated | 13a | 35a | 4 |
| Granular banded | 8b | 17ab | 6 |
| Granular seed-placed | 10ab | 21ab | 5 |
| *ANOVA* | | *Probability* | |
| Block | 0.5415 | 0.4003 | 0.3789 |
| Treatment | 0.0126 | 0.0309 | 0.7752 |

**Second Production Year (2001):**

Nodulation was not scored in 2001. The extremely dry soils combined with the deep roots of the third year stand made collecting intact roots systems impossible. The low productivity that was observed at the CLC site in the establishment year for the pre-inoculated, on-site inoculated and granular seed placed treatments (Table 3) was observed again in the second production season (Table 8). In particular the on-site inoculated treatment at this site yielded very poorly for both the first and second cuts of 2001. In the establishment year the low productivity was the result of poor stand establishment. In 2000 when sufficient water was available, it appears as though the few established plants were very productive. However, during 2001, under water deficit, the few established plants were much less productive. The higher stand establishment at the Seager Wheeler and Tisdale sites seemed to support higher productivity during the drought year. However, even though productivity was reduced at the CLC site, this site supported the higher N-fixation rates. Although there were fewer plants on the on-site inoculated plots, the plants that were there had comparable $N_2$-fixation rates, to plants inoculated with the other formulations. Furthermore all of inoculated alfalfa treatments had higher %Ndfa than the uninoculated controls at this site. All of the treatments at the CLC supported higher %Ndfa than the comparable treatments at the other sites.

Weed counts at all of the sites were higher in 2001 (Table 9) than in the other two years (Tables 4 & 6), probably a direct result of the drought encouraging the opportunistic nature of weeds. None of the inoculation treatments in 2001 showed statistically significant differences in weed populations at any of the sites (Table 9). However, there was a tendency for both broadleaf and grassy weeds to be more abundant in the on-site inoculated treatments at the CLC site and may have contributed to the low productivity associated with this treatment at this site.

**Table 8.** Productivity in the second production year (2001) for alfalfa inoculated with different *Rhizobium* formulations.

| Treatment | CLC | SW | Tisdale | CLC | SW | Tisdale |
|---|---|---|---|---|---|---|
| | First cut biomass ($g/m^2$) | | | Second cut biomass ($g/m^2$) | | |
| Control | 227a | 209b | 171 | 170a | 62 | 116 |
| Pre-inoculated | 112b | 214ab | 177 | 131ab | 59 | 159 |
| On-site inoculated | 67c | 229a | 160 | 73b | 60 | 141 |
| Granular banded | 217a | 233a | 180 | 177a | 60 | 164 |
| Granular seed-placed | 158ab | 216ab | 123 | 181a | 68 | 112 |
| *ANOVA* | | *Probability* | | | *Probability* | |
| Block | 0.762 | <0.001 | 0.684 | 0.159 | 0.417 | 0.530 |
| Treatment | 0.001 | 0.024 | 0.197 | 0.016 | 0.988 | 0.152 |
| | First cut %ndfa[1] | | | Second cut %ndfa[1] | | |
| Control | 66.3b | 44.0 | 74.2 | 73.8b | 40.7 | 54.1 |
| Pre-inoculated | 79.9a | 39.0 | 57.4 | 85.0a | 40.3 | 57.3 |
| On-site inoculated | 91.6a | 58.2 | 78.4 | 92.1a | 55.8 | 58.3 |
| Granular banded | 79.0a | 54.5 | 61.5 | 80.8ab | 59.8 | 61.4 |
| Granular seed-placed | 90.4a | 46.5 | 62.3 | 83.1a | 42.7 | 62.2 |
| *ANOVA* | | *Probability* | | | *Probability* | |
| Block | 0.402 | 0.755 | *0.690* | 0.090 | <0.001 | 0.684 |
| Treatment | 0.006 | 0.303 | 0.159 | 0.016 | 0.054 | 0.150 |

**Table 9.** Weed densities in the second production year (2001) of alfalfa plots inoculated with different *Rhizobium* formulations.

| Treatment | CLC | SW | Tisdale |
|---|---|---|---|
| | ------ ---- Broadleaf weeds (no./m$^2$) ---------- | | |
| Control | 15 | 11 | 8 |
| Pre-inoculated | 17 | 12 | 9 |
| On-site inoculated | 20 | 15 | 10 |
| Granular banded | 10 | 14 | 10 |
| Granular seed-placed | 11 | 10 | 13 |
| *ANOVA* | | *Probability* | |
| Block | 0.3662 | 0.0086 | 0.1856 |
| Treatment | 0.2842 | 0.7632 | 0.8399 |
| | ---------- Grassy weeds (no./m$^2$) ---------- | | |
| Control | 5 | 9 | 6 |
| Pre-inoculated | 5 | 13 | 6 |
| On-site inoculated | 8 | 11 | 7 |
| Granular banded | 6 | 15 | 7 |
| Granular seed-placed | 4 | 10 | 7 |
| *ANOVA* | | *Probability* | |
| Block | 0.8992 | 0.4214 | 0.8863 |
| Treatment | 0.6514 | 0.2799 | 0.8671 |
| | ---------- Total weeds (no./m$^2$) ---------- | | |
| Control | 20 | 20 | 14 |
| Pre-inoculated | 22 | 25 | 15 |
| On-site inoculated | 28 | 26 | 17 |
| Granular banded | 16 | 29 | 17 |
| Granular seed-placed | 15 | 20 | 20 |
| *ANOVA* | | *Probability* | |
| Block | 0.5873 | 0.7689 | 0.5877 |
| Treatment | 0.0633 | 0.0908 | 0.6853 |

**Growth Chamber Experiment:**

Inoculant placement did not affect root or shoot productivity for either cultivar of alfalfa at any of the three sampling times (Table 10). The only statistical differences observed were between the two cultivars. As the experiment progressed (12 week sampling) the fibrous rooted Rambler alfalfa produced more root biomass than the tap-rooted Beaver alfalfa. Overall, shoot biomass was also higher for the Rambler alfalfa. There was some tendency for the granular-seed placed inoculant to enhance production of the roots in both cultivar ($P<0.01$). Nodules were removed from the root system during scoring, so this represents and actual increase in root mass, and is not due to the production of large nodules by plants in this treatment.

In addition, nodulation, nitrogen fixation, and the N content of plants inoculated with the different placements of the *Rhizobium* inoculants were not different (Table 11). All of the treatments showed very high N-fixation percentages under these controlled and near optimal conditions. As was the case in the field study uninoculated control plants also showed N fixation levels comparable to the inoculation treatments. Nitrogen fixation levels were higher in the greenhouse than in the establishment year of the field study. The field soils used in the greenhouse study were extremely deficient in N, containing 14 kg/ha available N. Furthermore, unlike in a field environment, in a pot experiment the roots can only access the volume of soil in the pot. Once this N is used up, the plant must rely on N-fixation to a greater extent. This combined with the near optimal temperatures and water regimes probably account for the higher N-fixation levels in the greenhouse than in the field.

Nodulation scores were higher at the 4 week sampling than the 8 or 12 week sampling even though N-fixation did not decrease with the age of the plant (Table 11). There were general differences in the placement of the nodules on the root systems of the plants. Nodules in the peat inoculated treatment tended to be located throughout the top of the root system with some concentration in the crown; nodules in the granular seed placed treatment tended to be distributed fairly evenly throughout the root system; and nodules in the granular inoculant treatment placed below the seed, regardless of depth of placement, tended to be distributed throughout the bottom portion of the root system.

There is some evidence that in pulse crops nodules situated on the lateral roots are more effective at fixing N than nodules in close proximity to the seed and tap root. Kyei-Boahen et al. (2002) reported a strong correlation between both seed yield and the amount of seed N fixed and weight of lateral root nodules. In this controlled environment study, there is no evidence that nodule placement affected $N_2$-fixation. However, in a field situation where conditions are not optimal, nodule placement may become important. In older stands, nodules located at depth associated with the newest roots to develop may contribute the most to N-fixation in the crop.

Beta-carotene values were not different among any of the treatments and hence, inoculation had no affect on this quality parameter. β-carotene is of particular importance as a quality indicator in dehy-alfalfa production, but is not generally considered for alfalfa hay production. It has been suggested to be a factor in fertility in horses (Agriculture Canada).

**Table 10.** Dry weights for alfalfa plants grown with granular inoculant placed at different depths from the seed in a growth chamber experiment. Each value is the mean of 4 replicates of 8 plants grown per pot.

| | *Beaver* | | | | | | |
|---|---|---|---|---|---|---|---|
| | 4 weeks | | 8 weeks | | 12 weeks | | |
| | shoot | root | shoot | root | shoot | root | β-carotene |
| | g pot$^{-1}$ | | | | | | μg g$^{-1}$ |
| Uninoculated | 2.8 | 1.6 | 4.4 | 3.3 | 5.2 | 4.6 | 88 |
| Peat-based | 3.3 | 1.9 | 4.2 | 3.2 | 5.1 | 4.3 | 86 |
| Granular-seed placed | 3.5 | 2.8 | 4.8 | 5.0 | 5.0 | 7.7 | 91 |
| Granular - 2.5 cm | 3.5 | 2.2 | 4.7 | 4.5 | 4.0 | 5.8 | 89 |
| Granular - 5.0 cm | 3.5 | 1.6 | 5.1 | 4.2 | 5.2 | 5.1 | 90 |
| | *Rambler* | | | | | | |
| Uninoculated | 2.6 | 1.9 | 4.8 | 4.3 | 4.9 | 6.6 | 87 |
| Peat-based | 2.9 | 1.7 | 4.9 | 4.9 | 5.6 | 6.6 | 91 |
| Granular-seed placed | 3.6 | 1.7 | 4.1 | 3.4 | 6.3 | 8.4 | 93 |
| Granular - 2.5 cm | 2.7 | 1.9 | 5.5 | 5.6 | 6.3 | 6.6 | 89 |
| Granular - 5.0 cm | 2.9 | 1.9 | 4.3 | 4.2 | 5.6 | 6.8 | 91 |
| *ANOVA* | *Probability* | | | | | | |
| Cultivar [C] | 0.131 | 0.419 | 0.805 | 0.905 | 0.011 | 0.022 | 0.873 |
| Treatment [T] | 0.348 | 0.610 | 0.740 | 0.255 | 0.759 | 0.093 | 0.656 |
| C × T | 0.733 | 0.302 | 0.313 | 0.083 | 0.154 | 0.895 | 0.448 |

**Table 11.** Nitrogen fixation for alfalfa plants grown with granular inoculant placed at different depths from the seed or with seed applied peat-based inoculant in a growth chamber experiment. Each value is the mean of 4 replicates of 8 plants grown per pot.

| | ------- 4 weeks ------- | | | ------- 8 weeks ------- | | | ------ 12 weeks ------ | | |
|---|---|---|---|---|---|---|---|---|---|
| | N content (%) | Ndfa (%) | Nod. Score | N content (%) | Ndfa (%) | Nod. Score | N content (%) | Ndfa (%) | Nod. Score |
| *Beaver* | | | | | | | | | |
| Uninoculated | 2.8 | 86.9 | 3.25 | 2.8 | 87.9 | 2.00 | 2.5 | 89.2 | 2.25 |
| Peat-based | 2.9 | 86.9 | 4.25 | 2.7 | 84.2 | 3.50 | 2.8 | 89.4 | 2.50 |
| Granular-seed placed | 2.6 | 91.0 | 4.50 | 2.9 | 89.9 | 3.50 | 2.9 | 93.1 | 2.25 |
| Granular - 2.5 cm | 2.7 | 90.6 | 4.67 | 2.8 | 88.8 | 2.50 | 2.8 | 91.6 | 2.25 |
| Granular - 5.0 cm | 2.5 | 89.6 | 4.00 | 2.7 | 89.0 | 2.50 | 2.7 | 91.3 | 2.25 |
| *Rambler* | | | | | | | | | |
| Uninoculated | 2.5 | 86.4 | 4.25 | 2.7 | 90.1 | 2.00 | 2.4 | 89.0 | 1.75 |
| Peat-based | 2.3 | 89.4 | 4.75 | 3.0 | 87.5 | 1.75 | 2.4 | 91.1 | 2.25 |
| Granular-seed placed | 2.5 | 90.1 | 4.75 | 2.9 | 88.4 | 1.75 | 2.4 | 92.9 | 2.00 |
| Granular - 2.5 cm | 2.3 | 87.8 | 4.50 | 2.7 | 89.7 | 2.25 | 2.3 | 90.0 | 2.75 |
| Granular - 5.0 cm | 2.5 | 92.4 | 4.00 | 2.9 | 88.2 | 2.00 | 2.4 | 93.9 | 1.75 |
| *ANOVA* | ---------- *Probability* ---------- | | | | | | | | |
| Cultivar [C] | <0.001 | 0.741 | 0.844 | 0.211 | 0.395 | 0.632 | 0.035 | 0.657 | 0.771 |
| Treatment [T] | 0.518 | 0.091 | 0.362 | 0.167 | 0.590 | 0.448 | 0.281 | 0.077 | 0.633 |
| C × T | 0.460 | 0.513 | 0.556 | 0.098 | 0.270 | 0.633 | 0.081 | 0.660 | 0.477 |

**Conclusions**

Granular *Rhizobium* inoculants were not generally superior to the traditional pre-inoculated seed nor on-site applied peat-based inoculants. There was some evidence that the granular formulations banded below the seed row resulted in higher yields in the establishment year, but this advantage disappeared by the second and third years. The higher yields were due to stronger stand establishment (more plants per area) rather than to superior N-fixation. The growth chamber study revealed that the depth of placement of the granules did not affect N-fixation even though generally the region of the root that was nodulated was different depending on the type of inoculant and its placement. Nodules located on lateral roots have been demonstrated to contribute more N from fixation to chickpea seed (Kyei-Boahen, 2002). It would seem that nodules on lateral roots develop later than nodules associated with the crown or tap roots and hence contribute to fixation when the need for N is high (i.e., during seed set). However, because the alfalfa in this study was not used for seed production, it seems that the time of nodule development has little effect on overall N-fixation.

It appears that granular inoculants provide no advantage in alfalfa production. However, if enhanced productivity in the establishment year turns out to be a general trend associated with the use of granular formulations, they might prove economically useful for the production of biennial forage legumes like sweet clover. Furthermore, they might provide a means of inoculating a crop post-emergently in the case of inoculant failure or simply failure to inoculate.

An area supported by this study that remains elusive is determining the factors that affect successful inoculation by indigenous rhizobia. In this study, in general, uninoculated controls were as productive and as effective at nodulating and fixing N as the plots inoculated with the various inoculant formulations. This was observed in both the field study and the growth chamber study. Furthermore, many alfalfa producers do not inoculate alfalfa and achieve excellent productivity. At the same time, there are consistently reports of inoculant failure or low productivity because of not inoculating. Determining the factors that affect the success of indigenous inoculation, or developing an assay for determining the likelihood of success of applying a commercial inoculant are areas that require further study.

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K. Hunter – technical assistance ~ 1 month

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